

# High affinity binding of proteins HMG I and HMG2 to semicatenated DNA loops

Claire Gaillard<sup>1</sup> and François Strauss<sup>1</sup>

Address: <sup>1</sup>Institut Jacques Monod, 2 Place Jussieu, 75251 Paris 05, France.

E-mail: <sup>1</sup> [gaillard@ijm.jussieu.fr](mailto:gaillard@ijm.jussieu.fr) <sup>1</sup> [strauss@ijm.jussieu.fr](mailto:strauss@ijm.jussieu.fr)

Published: 18 October 2000

Received: 11 August 2000

*BMC Molecular Biology* 2000, **1**:1

Accepted: 9 October 2000

The electronic version of this article is the complete one and can be found online at <http://www.biomedcentral.com/1471-2199/1/1>

## Abstract

**Background:** Proteins HMG I and HMG2 are two of the most abundant non histone proteins in the nucleus of mammalian cells, and contain a domain of homology with many proteins implicated in the control of development, such as the sex-determination factor Sry and the Sox family of proteins. In vitro studies of interactions of HMG1/2 with DNA have shown that these proteins can bind to many unusual DNA structures, in particular to four-way junctions, with binding affinities of  $10^7$  to  $10^9$  M<sup>-1</sup>.

**Results:** Here we show that HMG I and HMG2 bind with a much higher affinity, at least 4 orders of magnitude higher, to a new structure, Form X, which consists of a DNA loop closed at its base by a semicatenated DNA junction, forming a DNA hemicatenane. The binding constant of HMG I to Form X is higher than  $5 \times 10^{12}$  M<sup>-1</sup>, and the half-life of the complex is longer than one hour in vitro.

**Conclusions:** Of all DNA structures described so far with which HMG I and HMG2 interact, we have found that Form X, a DNA loop with a semicatenated DNA junction at its base, is the structure with the highest affinity by more than 4 orders of magnitude. This suggests that, if similar structures exist in the cell nucleus, one of the functions of these proteins might be linked to the remarkable property of DNA hemicatenanes to associate two distant regions of the genome in a stable but reversible manner.

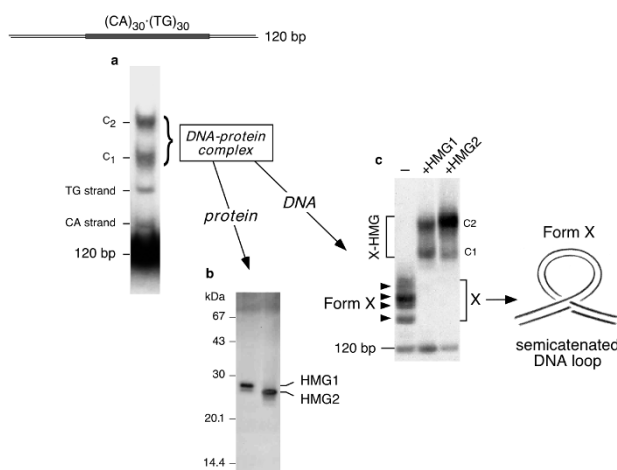
## Background

Proteins HMG1 and HMG2, two of the most abundant non histone proteins, have been known for more than 25 years (for a review see [1]), and their function has been the subject of varied investigations, especially since it was found that they contain a domain of homology with many proteins implicated in the control of development or of differentiation. As examples of recent studies are their immunocytochemical localization [2], the deletion of the gene coding for HMG1 by homologous recombination in transgenic mice [3], the effect of HMG1 or of the HMG domain on the assembly of certain nucleoprotein complexes [4], the observation of the binding of HMG1 to Oct and Hox proteins [5,6], to nuclear hormone recep-

tors [7], or to p53 [8], the influence of HMG1 on the circularization of short DNA fragments [9,10], on V(D)J rearrangement in vitro [11,12], or on transcription [13,14]. HMG1 has also been recently implicated as a mediator of endotoxin lethality [15]. An important way of studying the function and the mechanism of action of proteins HMG1/2 has obviously been the search for molecular partners, and, starting from the assumption that these chromatin proteins probably interact with DNA, many studies have been performed to study the sequences or the DNA conformations with which the proteins interact preferentially. For some HMG-domain proteins, specific binding sequences have been identified [16], but no such sequences have been found for proteins HMG1

and HMG2 themselves, which interact only weakly with double-stranded DNA. However, it has been shown that HMG1/2 could form complexes with several kinds of non-classical DNA structures: supercoiled circles [17,18,19,20], platinated DNA [21,22], UV-modified DNA [23], bulge loops [18,24,25], and four-way junctions [26]. As the interactions with four-way junctions were stronger than others, they have been the object of particular studies [18,26,27,28,29,30,31]. Thus, the image of HMG1 that has prevailed is of "an all-purpose DNA-bending, -wrapping, and -looping factor that can be recruited for transcription, DNA repair, and recombination" [32].

In the course of our studies with CA microsatellites, we have observed that a protein present in nuclear extracts of cultured monkey cells formed specific retarded complexes with a DNA fragment containing a tract of the poly(CA)<sub>i</sub> poly(TG)<sub>j</sub> sequence [33] (Fig. 1A). The purification of the DNA-binding activity yielded two proteins which were identified as HMG1 and HMG2 [33] (Fig. 1B). And the DNA contained in the complexes was present as a new form ("Form X", [33]), with a mobility lower than the regular double-stranded fragment, which was bound by purified HMG1 and HMG2 [33] (Fig. 1C) and which has been identified [34] as the semicatenated DNA loop which is schematically represented on the Figure. This structure consists in a double-stranded DNA loop at the base of which DNA duplexes cross and form a knot in which one of the strands of one duplex passes between the two strands of the other duplex, and reciprocally.



**Figure 1**  
Interaction of proteins HMG1 and HMG2 with Form X. (A) It was previously observed that a double-stranded 120 bp DNA fragment containing a 60 bp tract of the repetitive sequence poly(CA)<sub>i</sub> poly(TG)<sub>j</sub>, when end-labelled and used in a gel retardation experiment with a nuclear extract of cultured mon-

key cells (CV1 line) in the presence of high amounts of *E. coli* competitor DNA, could give rise to two retarded bands, C1 and C2, corresponding to specific DNA-protein complexes [33]. (B) The proteins responsible for the formation of retarded bands C1 and C2 were purified and identified as proteins HMG1 and HMG2 [33]. (C) The DNA contained in retarded bands C1 and C2 was purified: on a polyacrylamide gel it migrated more slowly than the regular double-stranded fragment and showed a series of bands, initially named "Form X", which reformed complexes C1 and C2 by interaction with HMG1/2, and which have been identified [34] as DNA loops maintained at their base by a semicatenated DNA junction. A highly schematic representation of the structure of Form X is shown, showing the junction in which two DNA duplexes cross with one of the strands of one duplex passing between the two strands of the other duplex, and reciprocally.

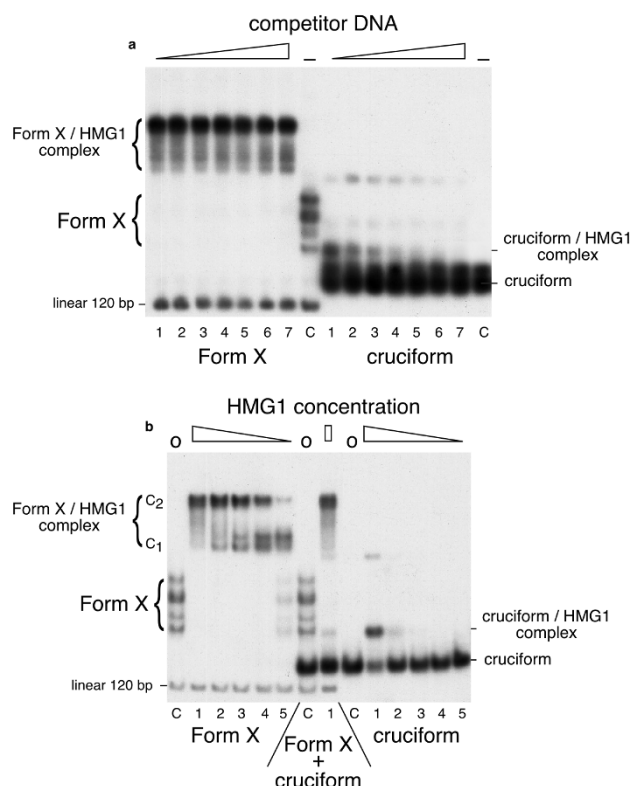
Here we have studied the interactions of HMG1/2 with Form X, and found that these proteins bind much more strongly to semicatenated DNA junctions than to any other known DNA substrate.

## Results

As HMG1 and HMG2 were known to interact with many different forms of DNA, we first compared the interactions of HMG1/2 with Form X and with several different DNA structures. Using labelled DNA minicircles as a substrate we observed preferential interactions of certain supercoiled topoisomers with HMG1/2 as described, but these interactions were always much weaker than the interactions with Form X (data not shown). Similarly, the interactions of HMG1/2 with bulge loops made by association of appropriate synthetic oligonucleotides were much weaker than the interactions of HMG1/2 with Form X. In all experiments with minicircles or bulge loops we could only observe a partial retardation of the labelled substrate and *E. coli* DNA was always an effective competitor of the interactions (not shown). In contrast, under identical conditions, Form X was always entirely retarded, and *E. coli* DNA was not a competitor.

Four-way junctions constitute another well-known substrate for HMG1/2 binding. We compared the affinity of HMG1 to four-way junctions and to Form X. Figure 2 shows in two different ways that the affinity of HMG1 for Form X is several orders of magnitude higher than for cruciform DNA. In the first experiment (Fig. 2A) the formation of complexes was studied in the presence of increasing amounts of *E. coli* competitor DNA. By comparing the first points in each series (lanes 1) it is observed that under strictly identical conditions all Form X is shifted whereas only part of the cruciform is retarded. It is also observed that the progressive addition of *E. coli* competitor DNA has no effect on the formation of complexes with Form X, whereas the complexes with cruciform disappear progressively. Identical results are obtained when a DNA containing CA microsatellites such as human DNA, or even pure poly(CA)<sub>i</sub> poly(TG)<sub>j</sub>,

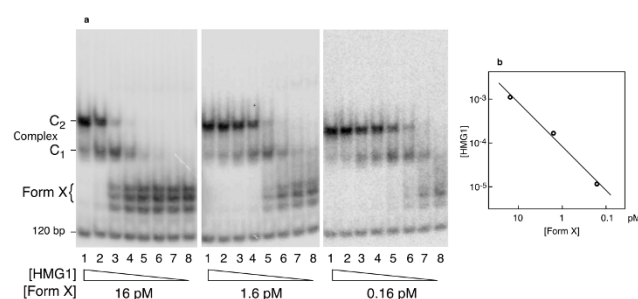
are used as competitor. A second experiment (Fig. 2B), with serial fivefold dilutions of protein HMG1 in the absence of competitor DNA, gives a similar result. With Form X, all the material is shifted except with dilution number 5. Complex C2 gradually disappears to give complex C1 when the HMG1 concentration decreases, suggesting that complexes C1 and C2 contain respectively one and two molecules of HMG1 bound per Form X. In contrast, with four-way junctions only 55% of the material is bound with the highest HMG concentration, and the retarded signal has already completely disappeared at the third concentration in the series. Mixing both substrates (two center lanes) confirms these observations. Identical results were obtained with protein HMG2.



**Figure 2**

Comparison of the interactions of HMG1 with Form X and cruciform. The concentrations used were: Form X:  $3.5 \times 10^{-11}$  M; cruciform:  $1.5 \times 10^{-10}$  M; undiluted HMG1:  $3.2 \times 10^{-8}$  M. (A) Form X (left) and cruciform (right) were labelled and incubated with a constant amount of HMG1 in the presence of increasing amounts of unlabelled *E. coli* competitor DNA. Lanes C are controls with no protein added. It is observed that Form X is entirely bound by HMG1 at all the competitor concentrations used. In contrast, cruciform is only partially bound in the first sample, and the amount of complex decreases quickly when the amount of competitor DNA is increased. Also note that linear DNA in its regular double-stranded form is not bound at all. (B) labelled Form X and cruciform were incubated in the presence of decreasing amounts of protein HMG1, with no addition of competitor DNA.

Lanes C contain controls with no protein added. The protein amounts are 10, 2, 0.4, 0.08, and 0.016 ng in lanes 1 to 5, respectively. Both central samples contain a mixture of Form X plus cruciform. To better compare the results with data published in the literature, the interactions and electrophoreses of the experiments shown in this Figure were strictly done under the conditions used in [26], 6.5% polyacrylamide gels in Tris-borate buffer, resulting in a change in mobility of Form X and of Form X-HMG1/2 complex as compared to experiments of Figures 1, 3 and 4, which were done in 4% polyacrylamide gels in Tris-acetate buffer. All the experiments shown here were also performed with HMG2, with identical results.



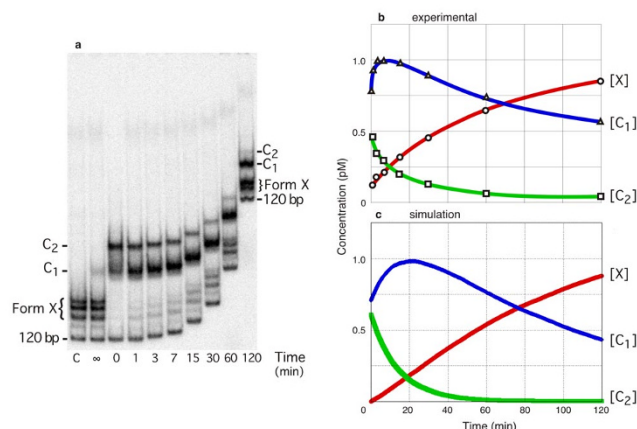
**Figure 3**

Interactions of Form X with variable concentrations of HMG1. Form X, end-labelled to the highest possible specific activity, was incubated with serial dilutions of protein HMG1. (A) The interactions were performed at three different concentrations of Form X (16 pM, 1.6 pM, and 0.16 pM). For each concentration the quantification of the radioactivity in the bands was performed with a phosphorimager, allowing us to determine the HMG1 concentration necessary to bind 50% of the Form X. (B) The results were plotted on a double logarithmic scale, showing that the protein concentration at half saturation (expressed as a dilution factor of a protein stock at  $\sim 20 \mu\text{g/mL}$  i.e.  $\sim 8 \times 10^{-7}$  M) decreases with the concentration of Form X in the samples, and therefore that the  $K_D$  of the interaction is lower than 0.16 pM.

Knowing the DNA and protein concentrations used, these experiments (Figs. 2A and 2B, lanes 1) lead to an estimate of the affinity constant of HMG1 for cruciform of about  $0.05 \times 10^9 \text{ M}^{-1}$  under the conditions used, in good agreement with values of  $0.01 \times 10^9$  to  $10^9$  which have been published or which can be deduced from published experiments for the interactions of HMG1 or of isolated HMG domains with cruciform [25,27,28]. To obtain an estimate of the much higher affinity constant of HMG1 for Form X, we had to lower the substrate concentration and use much higher dilutions of HMG1. This is shown in Figure 3, where we applied the serial dilution technique to estimate the affinity of HMG1 to Form X (for a review of the thermodynamic aspects of DNA-protein interactions, see e.g. [35,36]; for the determination of the parameters of interactions using the gel retardation technique, see [37]). The basis of the technique is that when 50% of the labelled substrate is shifted the law of mass action gives  $[P] = K_D + [D]/2$ , where  $[P]$  and  $[D]$  are the total protein and DNA concentrations, respec-

tively, and  $K_D$  is the dissociation constant, which is the reciprocal of the affinity constant. Thus, when concentrations are much higher than  $K_D$ , the amount of protein necessary to shift 50% of the DNA is proportional to the total DNA amount, whereas when the total DNA concentration becomes close to  $K_D$  the protein concentration necessary to bind 50% of the DNA tends to a constant value which is equal to  $K_D$ . Figure 3A shows the interactions of serial dilutions of HMG1 with decreasing concentrations of Form X: 16 pM, 1.6 pM and 0.16 pM (going much lower is difficult, the last concentration corresponding to 60 cpm of labelled DNA per sample). It is observed (Fig. 3B) that the amount of protein which is necessary to shift half of Form X decreases with the concentration of Form X, showing that even 0.16 pM is above  $K_D$ , and therefore that the affinity constant ( $1/K_D$ ) is higher than  $5 \times 10^{12} \text{ M}^{-1}$ , a very high value which is 4 orders of magnitude higher than the affinity constant of HMG1 to four-way junctions.

Such a high affinity constant is highly suggestive of a long half-life for the complex [35]. We estimated the stability of the complex by carrying out a competition with the substrate [37]. The complex between labelled Form X and HMG1 was preformed at a protein/DNA ratio of  $\sim 2$ . An excess of unlabelled Form X was then added and incubation was resumed. When the complex between labelled Form X and HMG1 dissociates, the released protein associates with the excess of unlabelled Form X, while the released Form X remains free. Equal volume aliquots of the mixture are loaded on a running polyacrylamide gel at different incubation times, thus freezing the dissociation process and allowing us to determine the amount of the initial (labelled) complex and the amount of free labelled Form X as a function of time (Fig. 4A). Measurement of the radioactivity in the bands was performed with a phosphorimager (Fig. 4B), and the comparison with a simulation done with the program Chemical Kinetics Simulator (Fig. 4C) yielded estimates for the time constant of dissociation of  $1.7 \times 10^{-4} \text{ s}^{-1}$  for complex C1 and  $1.2 \times 10^{-3} \text{ s}^{-1}$  for complex C2, corresponding to half-lives of more than one hour for complex C1 and about 10 min for complex C2. If it is assumed that complexes C1 and C2 correspond to complexes of Form X with one and two molecules of HMG1 respectively, the observed difference of stability between C1 and C2 might correspond to different localizations of HMG1 on Form X, for example outside or inside the loop. In agreement with this suggestion, Form X shows a series of different bands on gels, which correspond probably to differences in the size of the loop, localization of the junction along the DNA, and supercoiling inside the loop, and some of these bands are unable to form complex C2 (data not shown).



**Figure 4**

Stability of the complex between HMG1 and Form X as a function of time. (A) Complexes between labelled Form X and HMG1 were first formed during a 30 min incubation at  $37^\circ$  (lane 0, concentration of Form X =  $10^{-11} \text{ M}$ ; concentration ratio  $[\text{HMG1}]/[\text{FormX}] \cong 2$ ). At time 0, a 40 fold excess of unlabelled Form X was added and incubation at  $37^\circ$  was resumed. At each indicated time, from 1 min to 120 min, a sample was taken from the incubation mixture and immediately loaded on a running polyacrylamide gel, thus freezing the dissociation process. The curved shape of the autoradiogram is due to the fact that the first samples have migrated 4 hr while the last loaded samples have migrated 2 hr only. Controls: C, Form X with no protein added;  $\infty$ , competitor was added before the protein, which mimics complete protein redistribution after an incubation for an infinite time. (B) The radioactivity in the bands in (A) was counted with a phosphorimager and plotted as a function of time. Squares and green line: complex C2; triangles and blue line: complex C1; circles and red line: free Form X. (C) Best fit obtained by simulating the experiment with the program Chemical Kinetics Simulator, yielding estimates of for the dissociation time constants  $k_{\text{off}}$  equal to  $1.7 \times 10^{-4} \text{ s}^{-1}$  for complex C1 and  $1.2 \times 10^{-3} \text{ s}^{-1}$  for complex C2.

## Discussion

With our results showing that Form X is the strongest known DNA substrate for HMG1/2 proteins, the general situation with respect to the affinity of HMG1/2 to different DNA conformations can be summarized as follows: HMG1 and HMG2 have an affinity of the order of  $10^6$  for double-stranded DNA, bent or unbent, lower than the affinity of most HMG-domain proteins which have affinities high enough to show specific binding sites and complexes by gel retardation. A higher affinity is observed towards bulge loops, supercoiled circles, or platinated DNA. Then, with an affinity of  $10^8$  to  $10^9$ , the four-way junction was the known substrate of highest affinity for HMG1/2, and the suggestions of biological function for these proteins have most often dealt with such interactions. Now we observe a much tighter binding of HMG1 and HMG2 to Form X, with an affinity constant of more than  $5 \times 10^{12}$ , and the in vivo significance of interactions observed so far in vitro will have to be studied.

The first question is obviously whether structures similar to Form X exist in the cell, at what stages, and at which frequency. Technically, given the stability of Form X and its high affinity for HMG1/2, it should be possible to detect Form X in the cell even if it is rare. It will also be important to determine whether the HMG domain is as important in these interactions as with four-way junctions, and whether other HMG-domain proteins also bind Form X structures.

## Conclusions

Of all DNA structures described so far with which HMG1 and HMG2 interact, we have found that Form X, a DNA loop with a semicatenated DNA junction at its base, is the structure with the highest affinity by more than 4 orders of magnitude, suggesting that the possibility should be studied that such DNA hemicatenanes might exist in the cell nucleus. In addition, a role for the HMG domain in the formation or the stabilization of higher order chromatin structures has often been suggested (reviews in [1,32,38]), and our results go further in the same direction, by suggesting that one of the functions of HMG1/2 might be linked to the remarkable property of Form X to associate two distant regions of the genome in a stable but reversible manner.

## Materials and Methods

### Form X

The formation and purification of Form X has been described in detail elsewhere [34]. Briefly, a DNA fragment containing a tract of poly(CA)<sub>i</sub> poly(TG)<sub>j</sub> in its center is heat-denatured and allowed to renature in the presence of protein HMG1/2. The complexes formed, in which HMG1/2 is found associated with Form X, are purified by polyacrylamide gel electrophoresis and electroeluted. The protein is then removed by extraction with chloroform in 1 M NaCl and 1% SDS, Form X is ethanol precipitated and redissolved in 0.1 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 7.5.

Four-way junctions were prepared with synthetic oligonucleotides with sequences identical to those used in [26].

### Proteins HMG1 and HMG2

Proteins HMG1 and HMG2 were purified as described [33]. In the first experiments, their concentration was determined by electrophoresis on SDS-polyacrylamide gels, staining with coomassie blue, and comparison with known markers. Later, given the quantitative binding of HMG1 and HMG2 to Form X, a better precision was achieved by quantifying the interactions with known amounts of radioactively labelled Form X.

## Protein-DNA interactions

Interactions were performed in 25 µL of 25 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM EDTA, 1 mM DTT plus 100 µg/mL bovine serum albumin to prevent losses of material on tube walls (0.1% Triton X-100 is equally efficient). After addition of 1 µL of HMG1/2 protein, samples were incubated for 30 min. at 37° and loaded on a polyacrylamide gel. HMG and DNA concentrations are indicated in the Figure legends. Unless otherwise noted, electrophoresis was performed in 4% polyacrylamide gels (acrylamide:bis 29:1) in 6.7 mM Tris-acetate, 3.3 mM Na acetate, 1 mM EDTA, pH 7.8 at 4° with buffer recirculation.

## Acknowledgements

We would like to thank Nathalie Delgehr for her help with experiments with bulge loops, Caroline Perrin for technical assistance, and Susan Elsevier for critical reading of the manuscript. This work was made possible in part by grants from the Association Française contre les Myopathies, the Ligue Nationale Française Contre le Cancer, and the Association pour la Recherche contre le Cancer.

## References

1. Bustin M, Reeves R: **High-mobility-group chromosomal proteins: architectural components that facilitate chromatin function.** *Prog Nucleic Acid Res Mol Biol* 1996, **54**:35-100
2. Falciola L, Spada F, Calogero S, Langst G, Voit R, Grummt I, Bianchi ME: **High mobility group I protein is not stably associated with the chromosomes of somatic cells.** *J Cell Biol* 1997, **137**:19-26
3. Calogero S, Grassi F, Aguzzi A, Voigtlander T, Ferrier P, Ferrari S, Bianchi ME: **The lack of chromosomal protein HmgI does not disrupt cell growth but causes lethal hypoglycaemia in newborn mice.** *Nat Genet* 1999, **22**:276-280
4. Grosschedl R, Giese K, Pagel J: **HMG domain proteins: architectural elements in the assembly of nucleoprotein structures.** *Trends Genet* 1994, **10**:94-100
5. Zwilling S, König H, Wirth T: **High mobility group protein 2 functionally interacts with the POU domains of octamer transcription factors.** *Embo J* 1995, **14**:1198-1208
6. Zappavigna V, Falciola L, Citterich MH, Mavilio F, Bianchi ME: **HMG1 interacts with HOX proteins and enhances their DNA binding and transcriptional activation.** *Embo J* 1996, **15**:4981-4991
7. Boonyaratankornkit V, Melvin V, Prendergast P, Altmann M, Ronfani L, Bianchi ME, Taraseviciene L, Nordeen SK, Allegretto EA, Edwards DP: **High-mobility group chromatin proteins I and 2 functionally interact with steroid hormone receptors to enhance their DNA binding in vitro and transcriptional activity in mammalian cells.** *Mol Cell Biol* 1998, **18**:4471-4487
8. Jayaraman L, Moorthy NC, Murthy KG, Manley JL, Bustin M, Prives C: **High mobility group protein-I (HMG-I) is a unique activator of p53.** *Genes Dev* 1998, **12**:462-472
9. Paull TT, Haykinson MJ, Johnson RC: **The nonspecific DNA-binding and -bending proteins HMG1 and HMG2 promote the assembly of complex nucleoprotein structures.** *Genes Dev* 1993, **7**:1521-1534
10. Pil PM, Chow CS, Lippard SJ: **High-mobility-group I protein mediates DNA bending as determined by ring closures.** *Proc Natl Acad Sci U S A* 1993, **90**:9465-9469
11. van Gent DC, Hiom K, Paull TT, Gellert M: **Stimulation of V(D)J cleavage by high mobility group proteins.** *Embo J* 1997, **16**:2665-2670
12. Aidinis V, Bonaldi T, Beltrame M, Santagata S, Bianchi ME, Spanopoulou E: **The RAG1 homeodomain recruits HMG1 and HMG2 to facilitate recombination signal sequence binding and to enhance the intrinsic DNA-bending activity of RAG1-RAG2.** *Mol Cell Biol* 1999, **19**:6532-6542
13. Tremethick DJ, Molloy PL: **High mobility group proteins I and 2 stimulate transcription in vitro by RNA polymerases II and III.** *J Biol Chem* 1986, **261**:6986-6992

14. Sutrias-Grau M, Bianchi ME, Bernues J: **High mobility group protein I interacts specifically with the core domain of human TATA box-binding protein and interferes with transcription factor IIB within the pre-initiation complex.** *J Biol Chem* 1999, **274**:1628-1634
15. Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A, Tracey KJ: **HMG-I as a late mediator of endotoxin lethality in mice.** *Science* 1999, **285**:248-251
16. Bianchi ME: **The HMG-box domain.** In: *DNA-proteins: structural interactions* (Edited by Lilley DMJ) IRL Press at Oxford University 1995,
17. Sheflin LG, Fucile NVV, Spaulding SVW: **The specific interactions of HMG I and 2 with negatively supercoiled DNA are modulated by their acidic C-terminal domains and involve cysteine residues in their HMG I/2 boxes.** *Biochemistry* 1993, **32**:3238-3248
18. Payet D, Travers A: **The acidic tail of the high mobility group protein HMG-D modulates the structural selectivity of DNA binding.** *J Mol Biol* 1997, **266**:66-75
19. Stros M, Reich J: **Formation of large nucleoprotein complexes upon binding of the high- mobility-group (HMG) box B-domain of HMG I protein to supercoiled DNA.** *Eur J Biochem* 1998, **251**:427-434
20. Yoshioka K, Saito K, Tanabe T, Yamamoto A, Ando Y, Nakamura Y, Shirakawa H, Yoshida M: **Differences in DNA recognition and conformational change activity between boxes A and B in HMG2 protein.** *Biochemistry* 1999, **38**:589-595
21. Pil PM, Lippard SJ: **Specific binding of chromosomal protein HMG I to DNA damaged by the anticancer drug cisplatin.** *Science* 1992, **256**:234-237
22. Locker D, Decoville M, Maurizot JC, Bianchi ME, Leng M: **Interaction between cisplatin-modified DNA and the HMG boxes of HMG I: DNase I footprinting and circular dichroism.** *J Mol Biol* 1995, **246**:243-247
23. Pasheva EA, Pashev IG, Favre A: **Preferential binding of high mobility group I protein to UV-damaged DNA. Role of the COOH-terminal domain.** *J Biol Chem* 1998, **273**:24730-24736
24. Gibb CL, Cheng W, Morozov VN, Kallenbach NR: **Effect of nuclear protein HMG I on in vitro slippage synthesis of the tandem repeat dTG x dCA.** *Biochemistry* 1997, **36**:5418-5424
25. Payet D, Hillisch A, Lowe N, Diekmann S, Travers A: **The recognition of distorted DNA structures by HMG-D: a footprinting and molecular modelling study.** *J Mol Biol* 1999, **294**:79-91
26. Bianchi ME, Beltrame M, Paonessa G: **Specific recognition of cruciform DNA by nuclear protein HMG I.** *Science* 1989, **243**:1056-1059
27. Ferrari S, Harley VR, Pontiggia A, Goodfellow PN, Lovell-Badge R, Bianchi ME: **SRY, like HMG I, recognizes sharp angles in DNA.** *Embo J* 1992, **11**:4497-4506
28. Falciola L, Murchie AI, Lilley DM, Bianchi M: **Mutational analysis of the DNA binding domain A of chromosomal protein HMG I.** *Nucleic Acids Res* 1994, **22**:285-292
29. Teo SH, Grasser KD, Hardman CH, Broadhurst RW, Laue ED, Thomas JO: **Two mutations in the HMG-box with very different structural consequences provide insights into the nature of binding to four-way junction DNA.** *Embo J* 1995, **14**:3844-3853
30. Hill DA, Pedulla ML, Reeves R: **Directional binding of HMG-I(Y) on four-way junction DNA and the molecular basis for competitive binding with HMG-I and histone H1.** *Nucleic Acids Res* 1999, **27**:2135-2144
31. Webb M, Thomas JO: **Structure-specific binding of the two tandem HMG boxes of HMG I to four- way junction DNA is mediated by the A domain.** *J Mol Biol* 1999, **294**:373-387
32. Bianchi ME, Beltrame M: **Flexing DNA: HMG-box proteins and their partners.** *Am J Hum Genet* 1998, **63**:1573-1577
33. Gaillard C, Strauss F: **Association of poly(CA).poly(TG) DNA fragments into four-stranded complexes bound by HMG I and 2.** *Science* 1994, **264**:433-436
34. Gaillard C, Strauss F: **DNA loops and semicatenated DNA junctions.** *BMC Biochem Struct Biol* 2000, **1**:1-
35. Berg OG, Winter RB, von Hippel PH: **How do genome-regulatory proteins locate their DNA target sites?** *Trends Biochem Sci* 1982, **7**:52-55
36. von Hippel PH, Berg OG: **On the specificity of DNA-protein interactions.** *Proc Natl Acad Sci U S A* 1986, **83**:1608-1612
37. Fried M, Crothers DM: **Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis.** *Nucleic Acids Res* 1981, **9**:6505-6525
38. Lilley DM: **DNA--protein interactions. HMG has DNA wrapped up.** *Nature* 1992, **357**:282-283